REMARKS

Initially, Applicants would like to thank the Examiner for the indication that claims 23, 24 and 27-33 contain allowable subject matter.

Claim 12 has been amended by deleting "bleaching" from the claim. Thus, claims 12, 13 and 16-22 relate to methods of depigmenting skin.

New claims 46-54 have been added. These new claims are directed to methods of bleaching skin, subject matter originally found in claims 12, 13 and 16-22. Accordingly, no new matter has been added through these amendments and new claims.

Claims 12, 13, 16-24, 27-33 and 36-54 are currently pending, although claims 36-45 have been withdrawn from consideration.

The Office Action rejected claims 12, 13, 16-19 and 22 under 35 U.S.C. § 102 as anticipated by U.S. patent 4,542,129 ("Orentreich") and/or U.S. patent 5,869,090 ("Rosenbaum"), and claims 12, 13, 16-22 under 35 U.S.C. § 103 as obvious over JP 07196467 ("Nobuo") and Orentreich in view of U.S. patent 5,776,438 ("Tokue"). In view of the following comments, Applicants respectfully request reconsideration and withdrawal of these rejections.

Regarding the § 102 rejections, it is undisputed that neither Orentreich nor Rosenbaum expressly discloses the claimed methods. The Office Action has attempted to compensate for Orentreich's and Rosenbaum's complete failure to disclose the claimed methods by asserting that the cited references inherently anticipate them. Central to this assertion is the belief that the same population of people would use both Orentreich's/Rosenbaum's methods and the claimed methods: that is, those practicing Orentreich's and Rosenbaum's methods would necessarily also want to improve the homogeneity of their skin. However, contrary to the Office Action's assertions, practicing

Orentreich's and Rosenbaum's methods do not inherently result in practicing the claimed methods.

As noted in the Background Section of the present application, human skin color depends on many factors. This fact is supported by Tabs A and B attached hereto (articles found on the Internet). It is also indisputable that many different types of skin color exist, resulting in a wide, wide variety of appearances worldwide. For example, the skin color of albinos is extremely light and severely deficient in melanin. As another example, some people have a tremendous amount of freckles, while others have no freckles at all. The bottom line is that human skin is extremely variable due to the numerous factors which go into coloration -- not all skin is alike, particularly when it comes to coloration.

Given this wide variation in skin appearance, no generalizations can be made concerning the effect which specific compositions might have on skin. In other words, it does not necessarily follow that applying a specific composition to skin will necessarily result in a specific outcome. Would the skin of an albino be bleached if a bleaching composition were applied to it? Would freckle-less skin be depigmented if a depigmenting composition were applied to it?

For Orentreich or Rosenbaum to inherently anticipate the invention methods, these references must necessarily result in depigmenting or bleaching human skin in need of such benefits. *See, Eli Lilly & Co. v. Barr Laboratories, Inc.*, 251 F.3d 955 (Fed. Cir. 2001)(inherent anticipation requires that the claimed invention necessarily result from the prior art disclosure); *Jansen v. Rexall Sundown Inc.*, 68 U.S.P.Q.2d 1154 ("in need thereof" language is not satisfied if the active ingredient is administered for a purpose other than the claimed purpose). In other words, the invention methods must naturally flow from Orentreich's or Rosenbaum's disclosure. *See, Eli Lilly, supra.* As noted in *In re Oelrich*, 666 F.2d 578, 581, 212 USPQ 323 (CCPA 1981), the mere fact that a certain thing may result

from a given set of circumstances is not sufficient to prove inherency: inherency may not be established by probabilities or possibilities.

Thus, something that is inherent must **inevitably** be the result **each and every time**. Here, neither Orentreich nor Rosenbaum inevitably leads to the claimed methods each and every time. Accordingly, neither reference can inherently anticipate the claimed methods.

Initially, Applicants note that the Office Action's assertion that "most people" would like to improve skin homogeneity (see, sentence spanning pages 6-7 of the Office Action) is legally insufficient for demonstrating that Orentreich's or Rosenbaum's methods inherently result in the claimed methods. As explained above, for such inherency to exist, practicing Orentreich's or Rosenbaum's methods would necessarily have to result in the claimed methods, meaning that each and every person would need to want to improve skin homogeneity for inherency to exist. Because only "most people" practicing Orentreich's or Rosenbaum's methods would want to improve skin homogeneity, practicing these methods cannot inherently result in the claimed methods as a matter of law. For at least this reason the inherency rejections are improper and should be withdrawn.

Moreover, Orentreich does not disclose actual application of one of the claimed DHEA compounds to humans. Rather, Orentreich demonstrates applying DHEA to hamsters, not humans. Thus, Orentreich cannot inherently disclose achieving the claimed benefits on humans, so Orentreich cannot inherently anticipate the claimed methods. Furthermore, Orentreich neither teaches nor suggests applying his compositions to human skin capable of being bleached or depigmented. Because not all skin is capable of being bleached or depigmented, Orentreich's non-descript disclosure cannot inherently lead to bleaching or depigmentation on skin in need thereof. For this reason as well, Orentreich cannot inherently anticipate the claimed methods.

Similarly, Rosenbaum neither teaches nor suggests applying his compositions to human skin capable of being bleached or depigmented, so Rosenbaum cannot inherently disclose achieving the claimed benefits on humans, meaning that it cannot inherently anticipate the claimed methods.

Finally, before the Patent Office can switch the burden of proof of showing non-inherency to Applicants, the Patent Office must provide some evidence or scientific reasoning to establish the reasonableness of the belief that the functional limitation is an inherent characteristic of the prior art. *See, Ex parte Skinner*, 2 USPQ2d 1788. In this case, the Patent Office has provided no such evidence, particularly in view of the wide, wide variation of skin coloration which exists. Accordingly, the inherency rejections are improper for this reason as well.

In view of the above, Applicants respectfully submit that the inherent anticipation rejections under 35 U.S.C. § 102 are improper and should be withdrawn.

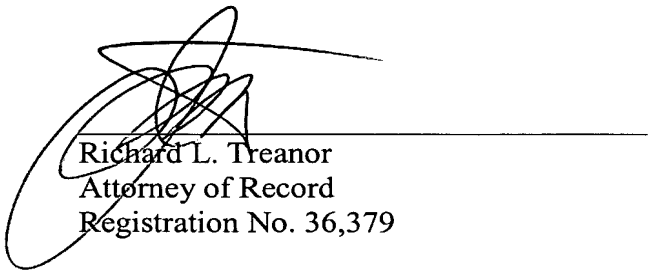
Regarding the § 103 rejection, neither Nobuo nor Tokue compensates for Orentreich's deficiencies. None of the cited references, alone or in combination, teach or suggest bleaching or depigmenting skin using the claimed DHEA compounds. In other words, based on the disclosures in the cited references, one skilled in the art would not have been motivated to use the claimed DHEA compounds to bleach or depigment skin with the expectation that the desired bleaching or depigmentation would have resulted.

In view of the above, Applicants respectfully submit that the rejection under 35 U.S.C. § 103 is improper and should be withdrawn.

Applicants believe that the present application is in condition for allowance. Prompt and favorable consideration is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



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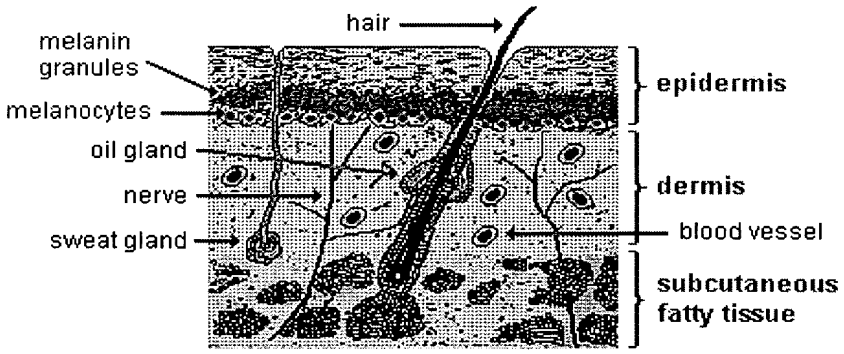
Skin Color Adaptation

Human skin color is quite variable around the world. It ranges from a very dark brown among some Africans, Australians, and Melanesians to a near yellowish pink among some Northern Europeans. There are no people who actually have true black, white, red, or yellow skin. These are commonly used color terms that do not reflect biological reality.

Some of the variation in human skin coloration
(Sub-Saharan African, Indian, Southern European, and Northern European)

Skin color is due primarily to the presence of a pigment called **melanin**. Both light and dark complexioned people have this pigment. However, two forms are produced--pheomelanin, which is red to yellow in color, and eumelanin, which is dark brown to black. People with light complexioned skin mostly produce pheomelanin, while those with dark colored skin mostly produce eumelanin. In addition, individuals differ in the number and size of melanin particles. The latter two variables are more important in determining skin color than the percentages of the different kinds of melanin. In lighter skin, color is also affected by red cells in blood flowing close to the skin. To a lesser extent, the color is affected by the presence of fat under the skin and carotene, a reddish-orange pigment in the skin.

Melanin is normally located in the epidermis, or outer skin layer. It is produced at the base of the epidermis by specialized cells called **melanocytes**.



Cross section of human skin
(colors are not true to life in this illustration)

Nature has selected for people with darker skin in tropical latitudes, especially in nonforested regions, where **ultraviolet radiation** from the sun is usually the most intense. Melanin acts as a protective biological shield against ultraviolet radiation. By

doing this, it helps to prevent sunburn damage that could result in DNA changes and, subsequently, **melanoma** --a cancer of the skin. Melanoma is a serious threat to life. In the United States, approximately 54,000 people get this aggressive type of cancer every year and nearly 8,000 of them die from it. Those at highest risk are European Americans. They have a 10 times higher risk than African Americans.

Ultraviolet radiation reaching the earth usually increases in summer and decreases in winter. The skin's ability to tan in summertime is an acclimatization to this seasonal change. Tanning is primarily an increase in the number and size of melanin granules due to the stimulation of ultraviolet radiation.

While skin tanning is often most noticeable on light complexioned people, even those with very dark brown skin can tan as a result of prolonged exposure to the sun. Some Northwest Europeans have substantially lost the ability to tan as a result of relaxed natural selection. Their skin burns and peels rather than tans. This is due to the fact that they produce a defective form of a skin protein (melanocortin-1 receptor or Mc1r) which is necessary for the production of melanin. They are at a distinct disadvantage in tropical and subtropical environments. Not only do they suffer the discomfort of readily burning, but they are at a much higher risk for skin cancer. The same is true of albinos.

Irish boy who is essentially unable to tan

It would be harmful if melanin acted as a complete shield. A certain amount of shortwave ultraviolet radiation (UVB) must penetrate the outer skin layer in order for the body to produce **vitamin D**. Approximately 90% of this vitamin in people normally is synthesized in their skin and the kidneys from a cholesterol-like precursor chemical with the help of ultraviolet radiation. The remaining 10% comes from foods such as fatty fish and egg yolks. Simple vitamin D is converted by our bodies into two sequential forms. The last form, commonly referred to as vitamin D_3, is needed for the intestines to absorb calcium and phosphorus from food for bone growth and repair. Calcium is also necessary in adults to maintain normal heart action, blood clotting, and a stable nervous system. Vitamin D plays an additional important role in promoting the production of cathelicidin, which apparently is an effective defender against fungal, bacterial, and viral infections, including the common flu.

Too much ultraviolet radiation penetrating the skin may cause the break down of folic acid (or folate--one of the B vitamins) in the body, which can cause anemia. Pregnant women who are deficient in folic acid are at a higher risk of having babies with neural tube defects. Because folic acid is needed for DNA replication in dividing cells, its absence can have an effect on many body processes, including the production of sperm cells. It may be that the ability to produce melanin was selected for in our early human ancestors because it helped preserve the body's folic acid supply in addition to reducing the chances of developing skin cancer.

People who live in far northern latitudes, where solar radiation is relatively weak most of the year, have an advantage if their skin has little shielding pigmentation. Nature selects for less melanin when ultraviolet radiation is weak. In such an environment, very dark skin is a disadvantage because it can prevent people from producing enough vitamin D, potentially resulting in rickets disease in children and osteoporosis in adults. Contributing

to the development of osteoporosis in older people is the fact that their skin generally loses some of its ability to produce vitamin D. Women who had prolonged vitamin D deficiencies as girls have a higher incidence of pelvic deformities that prevent normal delivery of babies.

The Inuit people of the American Subarctic are an exception. They have moderately heavy skin pigmentation despite the far northern latitude at which they live. While this is a disadvantage for vitamin D production, they apparently made up for it by eating fish and sea mammal blubber that are high in D. In addition, the Inuit have been in the far north for only about 5,000 years. This may not have been enough time for significantly lower melanin production to have been selected for by nature.

In the United States and other developed nations, milk is now usually fortified with vitamins D and A in order to prevent developmental problems such as those described above. However, the popularity of soft drinks and other alternatives to milk along with a decrease in the amount of time spent outdoors has led to a considerable rise in the rate of rickets disease. Not surprisingly, vitamin D deficiency is most acute in the winter in temperate and colder zones.

There is also a strong correlation between the amount of sunlight that children are exposed to and whether or not they will develop multiple sclerosis as adults. Most cases of this degenerative neural disorder are in the temperate regions of the world where the sunlight is rarely intense. Children growing up in tropical and subtropical regions rarely develop MS regardless of where their ancestors came from. This protection apparently continues for those who move to far northern or far southern regions after 16 years of age. What processes are responsible for this protection from MS and its possible relationship to skin color are unknown.

New research by Nina Jablonski and George Chaplin has led to the discovery that women generally produce 3-4% less melanin in their skin than do men in all populations of the world. They suggest that this is probably due to the fact that women have far higher calcium requirements during their reproductive years. Mate selection preference and other cultural practices may also be partly responsible for this gender difference in skin coloration.

Skin Color Distribution Around the World

Before the mass global migrations of people during the last 500 years, dark skin color was mostly concentrated near the equator and light color progressively increased further away, as illustrated in the map below. In fact, the majority of dark pigmented people lived within 20° of the equator. Most of the lighter pigmented people lived in the northern hemisphere north of 20° latitude.

(Data for native populations collected by R. Biasutti prior to 1940.)

Such a non-random distribution pattern of human skin color was predicted by **Wilhelm Gloger**, a 19th century naturalist. In 1833, he observed that heavily pigmented animals are to be found mostly in hot climates where there is intense sunshine. Conversely, those in cold climates closer to the poles commonly have light pigmentation. The relative intensity of solar radiation is largely responsible for this distribution pattern.

There are exceptions to **Gloger's rule** in the animal kingdom. In some cases, these are due to the fact that the survival value of having a camouflaged body can be more important than the selective pressures of ultraviolet radiation. Among humans, mate selection preferences may counter some of the evolutionary trend in skin color predicted by Gloger. The Inuit case described earlier suggests that diet may also be a significant factor in some societies. In the United States today, milk is regularly fortified with vitamin D to reduce the likelihood of children having calcium deficiencies. Despite this effort, some segments of the population still have high rates of calcium deficiency--especially African Americans and the elderly.

NEWS: In the April 2001 issue of the journal Pediatrics, there is a report concerning malnutrition among children in the U.S. state of Georgia that indicates there is a high frequency of rickets disease, especially among African Americans. This previously rare condition, which is caused by vitamin D deficiency, is making a comeback. There are now about 200,000 cases of it in Georgia. The study suggests that the dramatic increase in frequency is mainly due to three things: drinking milk substitutes that do not contain vitamin D, the failure to supplement breast milk, and insufficient exposure to sunlight. The popularity of carbonated soft drinks may also contribute to the problem because they usually contain phosphoric acid which can hinder bone growth.

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Unsolved Mystery

What Controls Variation in Human Skin Color?

Gregory S. Barsh

Diversity of human appearance and form has intrigued biologists for centuries, but nearly 100 years after the term "genetics" was coined by William Bateson in 1906, the genes that underlie this diversity are an unsolved mystery. One of the most obvious phenotypes that distinguish members of our species, differences in skin pigmentation, is also one of the most enigmatic. There is a tremendous range of human skin color in which variation can be correlated with climates, continents, and/or cultures, yet we know very little about the underlying genetic architecture. Is the number of common skin color genes closer to five, 50, or 500? Do gain- and loss-of-function alleles for a small set of genes give rise to phenotypes at opposite ends of the pigmentary spectrum? Has the effect of natural selection on similar pigmentation phenotypes proceeded independently via similar pathways? And, finally, should we care about the genetics of human pigmentation if it is only skin-deep?

Unsolved Mysteries discuss a topic of biological importance that is poorly understood and in need of attention.

Why Should We Care?

From a clinical perspective, inadequate protection from sunlight has a major impact on human health (Armstrong et al. 1997; Diepgen and Mahler 2002). In Australia, the lifetime cumulative incidence of skin cancer approaches 50%, yet the oxymoronic "smart tanning" industry continues to grow, and there is controversy over the extent to which different types of melanin can influence susceptibility to ultraviolet (UV) radiation (Schmitz et al. 1995; Wenczl et al. 1998). At the other end of the spectrum, inadequate exposure to sunlight, leading to vitamin D deficiency and rickets, has been mostly cured by nutritional advances made in the early 1900s. In both cases, understanding the genetic architecture of human skin color is likely to provide a greater appreciation of underlying biological mechanisms, much in the same way that mutational hotspots in the gene *TP53* have helped to educate society about the risks of tobacco (Takahashi et al. 1989; Toyooka et al. 2003).

From a basic science perspective, variation in human skin color represents an unparalleled opportunity for cell biologists, geneticists, and anthropologists to learn more about the biogenesis and movement of subcellular organelles, to better characterize the relationship between genotypic and phenotypic diversity, to further investigate human origins, and to understand how recent human evolution may have been shaped by natural selection.

The Color Variation Toolbox

Historically, measurement of human skin color is often based on subjective categories, e.g., "moderate brown, rarely burns, tans very easily." More recently, quantitative methods based on reflectance spectrophotometry have been applied, which allow reddening caused by inflammation and increased hemoglobin to be distinguished from darkening caused by increased melanin (Alaluf et al. 2002b; Shriver and Parra 2000; Wagner et al. 2002). Melanin itself is an organic polymer built from oxidative tyrosine derivatives and comes in two types, a cysteine-rich red–yellow form known as pheomelanin and a less-soluble black–brown form known as eumelanin (Figure 1A). Discriminating among pigment types in biological samples requires chemical extraction, but is worth the effort, since the little we do know about common variation in human pigmentation involves pigment type-switching. The characteristic phenotype of fair skin, freckling, and carrot-red hair is associated with large amounts of pheomelanin and small amounts of eumelanin and is caused by loss-of-function alleles in a single gene, the melanocortin 1 receptor *(MC1R)* (Sturm et al. 1998; Rees 2000) However, *MC1R* variation has a significant effect on pigmentation only in populations where red hair and fair skin are common (Rana et al. 1999; Harding et al. 2000), and its primary effects—to promote eumelanin synthesis at the expense of pheomelanin synthesis, or vice versa—contribute little to variation of skin reflectance among or between major ethnic groups (Alaluf et al. 2002a).

More important than the ratio of melanin types is the total amount of melanin produced. In addition, histological characteristics of different-

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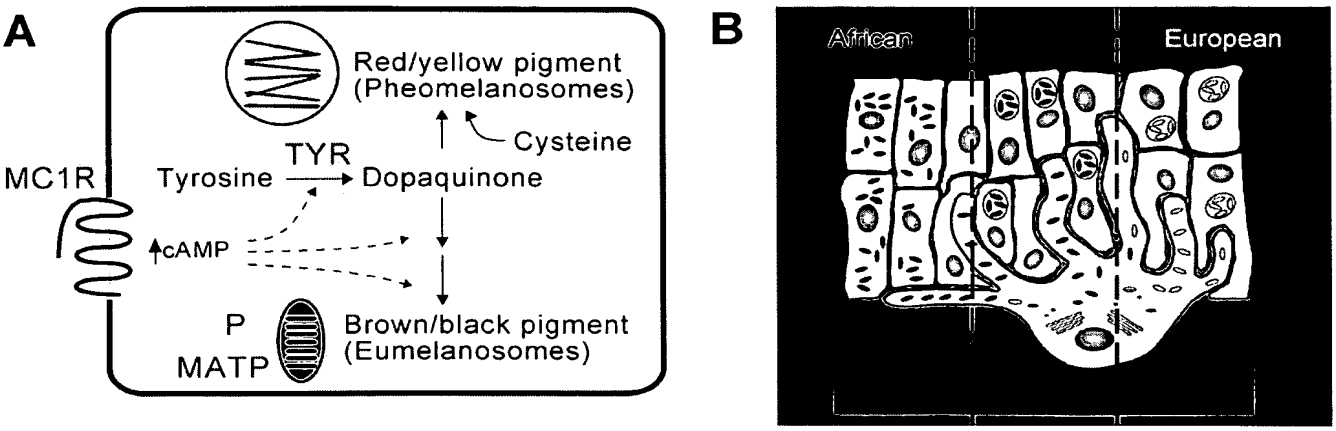

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Figure 1. Biochemistry and Histology of Different Skin Types

(A) Activation of the melanocortin 1 receptor (MC1R) promotes the synthesis of eumelanin at the expense of pheomelanin, although oxidation of tyrosine by tyrosinase (TYR) is required for synthesis of both pigment types. The membrane-associated transport protein (MATP) and the pink-eyed dilution protein (P) are melanosomal membrane components that contribute to the extent of pigment synthesis within melanosomes. (B) There is a gradient of melanosome size and number in dark, intermediate, and light skin; in addition, melanosomes of dark skin are more widely dispersed. This diagram is based on one published by Sturm et al. (1998) and summarizes data from Szabo et al. (1969), Toda et al. (1972), and Konrad and Wolff (1973) based on individuals whose recent ancestors were from Africa, Asia, or Europe.

colored skin provide some clues as to cellular mechanisms that are likely to drive pigmentary variation (Figure 1B). For the same body region, light- and dark-skinned individuals have similar numbers of melanocytes (there is considerable variation between different body regions), but pigment-containing organelles, called melanosomes, are larger, more numerous, and more pigmented in dark compared to intermediate compared to light skin, corresponding to individuals whose recent ancestors were from Africa, Asia, or Europe, respectively (Szabo et al. 1969; Toda et al. 1972; Konrad and Wolff 1973). From these perspectives, oxidative enzymes like tyrosinase (TYR), which catalyzes the formation of dopaquinone from tyrosine, or melanosomal membrane components like the pink-eyed dilution protein (P) or the membrane-associated transporter protein (MATP), which affect substrate availability and activity of TYR (Orlow and Brilliant 1999; Brilliant and Gardner 2001; Newton et al. 2001; Costin et al. 2003), are logical candidates upon which genetic variation could contribute to the diversity of human skin color.

Of equal importance to what happens inside melanocytes is what happens outside. Each pigment cell actively transfers its melanosomes to about 40 basal keratinocytes; ultimately, skin reflectance is determined by the amount and distribution of pigment granules within keratinocytes rather than melanocytes. In general, melanosomes of African skin are larger and dispersed more widely than in Asian or European skin (Figure 1). Remarkably, keratinocytes from dark skin cocultured with melanocytes from light skin give rise to a melanosome distribution pattern characteristic of dark skin, and vice versa (Minwalla et al. 2001). Thus, at least one component of skin color variation represents a gene or genes whose expression and action affect the pigment cell environment rather than the pigment cell itself.

Genetics of Skin Color

For any quantitative trait with multiple contributing factors, the most important questions are the overall heritability, the number of genes likely to be involved, and the best strategies for identifying those genes. For skin color, the broad sense heritability (defined as the overall effect of genetic vs. nongenetic factors) is very high (Clark et al. 1981), provided one is able to control for the most important nongenetic factor, exposure to sunlight.

Statements regarding the number of human skin color genes are attributed to several studies; one of the most complete is by Harrison and Owen (1964). In that study, skin reflectance measurements were obtained from 70 residents of Liverpool whose parents, grandparents, or both were of European ("with a large Irish component") or West African ("mostly from coastal regions of Ghana and Nigeria") descent and who were roughly classified into "hybrid" and "backcross" groups on this basis. An attempt to partition and analyze the variance of the backcross groups led to minimal estimates of three to four "effective factors," in this case, independently segregating genes. Aside from the key word *minimal* (Harrison and Owen's data could also be explained by 30–40 genes), one of the more interesting findings was that skin reflectance appeared to be mainly additive. In other words, mean skin reflectance of "F1 hybrid" or "backcross hybrid" groups is intermediate between their respective parental groups.

An alternative approach for considering the number of potential human pigmentation genes is based on mouse coat color genetics, one of the original models to define and study gene action and interaction, for which nearly 100 different genes have been recognized (Bennett and Lamoreux 2003; Jackson 1994). Setting aside mouse mutations that cause white spotting or predominant effects outside the pigmentary system, no

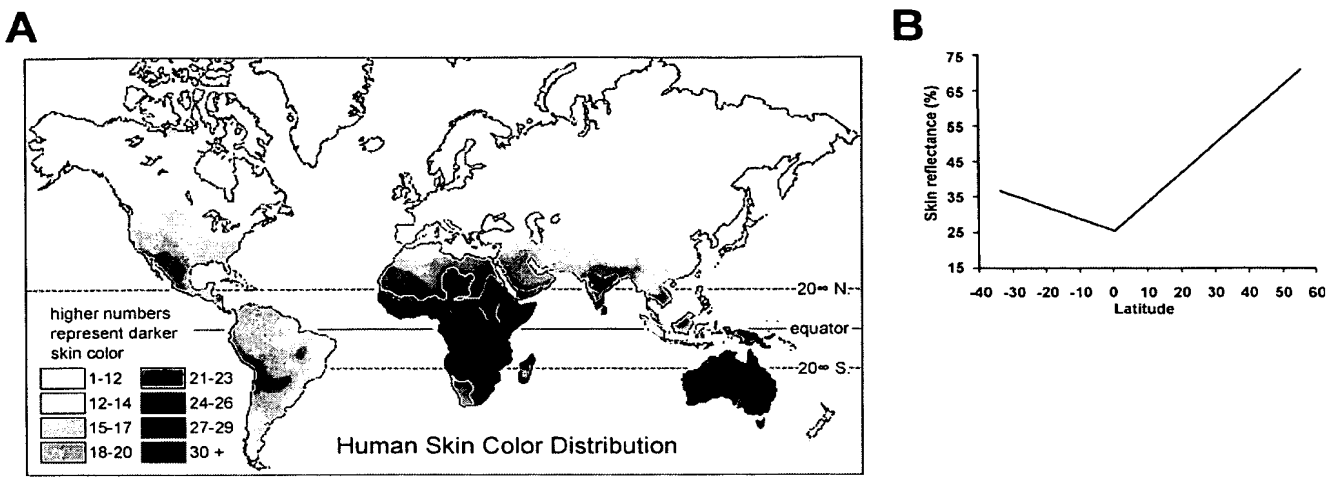

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Figure 2. Relationship of Skin Color to Latitude
(A) A traditional skin color map by Biasutti, based on http://anthro.palomar.edu/vary/. (B) Summary of 102 skin reflectance samples for males as a function of latitude, redrawn from Relethford (1997).

more than 15 or 20 mutations remain, many of which have been identified and characterized, and most of which have human homologs in which null mutations cause albinism.

This brings us to the question of candidate genes for skin color, since, like any quantitative trait, a reasonable place to start is with rare mutations known to cause an extreme phenotype, in this case Mendelian forms of albinism. The underlying assumption is that if a rare null allele causes a complete loss of pigment, then a set of polymorphic, i.e., more frequent, alleles with subtle effects on gene expression will contribute to a spectrum of skin colors. The *TYR*, *P*, and *MATP* genes discussed earlier are well-known causes of albinism whose primary effects are limited to pigment cells (Oetting and King 1999); among these, the *P* gene is highly polymorphic but the phenotypic consequences of *P* gene polymorphisms are not yet known.

Independent of phenotype, a gene responsible for selection of different skin colors should exhibit a population signature with a large number of alleles and rates of sequence substitution that are greater for nonsynonymous (which change an amino acid in the protein) than synonymous (which do not change any amino acid) alterations. Data have been collected only for *MC1R*, in which the most notable finding is a dearth of allelic diversity in African samples, which is remarkable given that polymorphism for most genes is greater in Africa than in other geographic regions (Rana et al. 1999; Harding et al. 2000). Thus, while *MC1R* sequence variation does not contribute significantly to variation in human skin color around the world, a functional *MC1R* is probably important for dark skin.

Selection for Skin Color?

Credit for describing the relationship between latitude and skin color in modern humans is usually ascribed to an Italian geographer, Renato Basutti, whose widely reproduced "skin color maps" illustrate the correlation of darker skin with equatorial proximity (Figure 2). More recent studies by physical anthropologists have substantiated and extended these observations; a recent review and analysis of data from more than 100 populations (Relethford 1997) found that skin reflectance is lowest at the equator, then gradually increases, about 8% per 10° of latitude in the Northern Hemisphere and about 4% per 10° of latitude in the Southern Hemisphere. This pattern is inversely correlated with levels of UV irradiation, which are greater in the Southern than in the Northern Hemisphere. An important caveat is that we do not know how patterns of UV irradiation have changed over time; more importantly, we do not know when skin color is likely to have evolved, with multiple migrations out of Africa and extensive genetic interchange over the last 500,000 years (Templeton 2002).

Regardless, most anthropologists accept the notion that differences in UV irradiation have driven selection for dark human skin at the equator and for light human skin at greater latitudes. What remains controversial are the exact mechanisms of selection. The most popular theory posits that protection offered by dark skin from UV irradiation becomes a liability in more polar latitudes due to vitamin D deficiency (Murray 1934). UVB (short-wavelength UV) converts 7-dehydrocholesterol into an essential precursor of cholecaliferol (vitamin D_3); when not otherwise provided by dietary supplements, deficiency for vitamin D causes rickets, a characteristic pattern of growth abnormalities and bony deformities. An oft-cited anecdote in support of the vitamin D hypothesis is that Arctic populations whose skin is relatively dark given their latitude, such as the Inuit and the Lapp, have had a diet that is historically rich in vitamin D. Sensitivity of modern humans to vitamin D deficiency is evident from the widespread occurrence of rickets in 19th-century industrial Europe, but whether dark-skinned humans migrating to polar latitudes tens or hundreds of thousands of years ago experienced similar problems is open to question. In any case, a risk for vitamin D deficiency can only explain

selection for light skin. Among several mechanisms suggested to provide a selective advantage for dark skin in conditions of high UV irradiation (Loomis 1967; Robins 1991; Jablonski and Chaplin 2000), the most tenable are protection from sunburn and skin cancer due to the physical barrier imposed by epidermal melanin.

Solving the Mystery

Recent developments in several areas provide a tremendous opportunity to better understand the diversity of human pigmentation. Improved spectrophotometric tools, advances in epidemiology and statistics, a wealth of genome sequences, and efficient techniques for assaying sequence variation offer the chance to replace misunderstanding and myths about skin color with education and scientific insight. The same approaches used to investigate traits such as hypertension and obesity—genetic linkage and association studies—can be applied in a more powerful way to study human pigmentation, since the sources of environmental variation can be controlled and we have a deeper knowledge of the underlying biochemistry and cell biology.

This approach is especially appealing given the dismal success rate in molecular identification of complex genetic diseases. In fact, understanding more about the genetic architecture of skin color may prove helpful in designing studies to investigate other quantitative traits. Current debates in the human genetics community involve strategies for selecting populations and candidate genes to study, the characteristics of sequence polymorphisms worth pursuing as potential disease mutations, and the extent to which common diseases are caused by common (and presumably ancient) alleles. While specific answers will be different for every phenotype, there may be common themes, and some answers are better than none.

Harrison and Owen concluded their 1964 study of human skin color by stating, "The deficiencies in the data in this study are keenly appreciated by the writers, but since there appear at present to be no opportunities for improving the data, it seems justifiable to take the analysis as far as possible." Nearly 40 years later, opportunities abound, and the mystery of human skin color is ready to be solved. ■

Acknowledgments

I am grateful to members of my laboratory and colleagues who study pigment cells in a variety of different experimental organisms for useful discussions and to Sophie Candille for helpful comments on the manuscript. Many of the ideas presented here emerged during a discussion series on Unsolved Mysteries in Biomedical Research that was initiated by Mark Krasnow and the Medical Scientist Training Program at Stanford University.

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Corrections

In *PLoS Biology*, volume 1, issue 1:

What Controls Variation in Human Skin Color?

Gregory S. Barsh
DOI: 10.1371/journal.pbio.0000027

The source of the image in Figure 2A was incorrectly acknowledged. The correct attribution is as follows:

(A) A traditional skin color map based on the data of Biasutti. Reproduced from http://anthro.palomar.edu/vary/ with permission from Dennis O'Neil.

The full text XML and HTML versions of the article have been corrected online. This correction note may be found online at DOI: 10.1371/journal.pbio.0000091.

Candidate Gene Association Study in Type 2 Diabetes Indicates a Role for Genes Involved in ß-Cell Function as Well as Insulin Action

Inês Barroso, Jian'an Luan, Rita P. S. Middelberg, Anne-Helen Harding, Paul W. Franks, Rupert W. Jakes, David Clayton, Alan J. Schafer, Stephen O'Rahilly, Nicholas J. Wareham
DOI: 10.1371/journal.pbio.0000020

One of the variants associated with increased diabetes risk was incorrectly indicated throughout this article. The A1369S variant in the gene *ABCC8* should have been written S1369A. The alanine variant is associated with increased risk. This mistake affects Tables 2 and 4, the text of the article in the section entitled *"ABCC8* and *KCNJ11"* on page 45, and the Supporting Information Tables S1 and S2.

The full text XML and HTML versions of the article, and the supporting Tables S1 and S2 have been corrected online. This correction note may be found online at DOI: 10.1371/journal.pbio.0000092.